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Mycoflora of Potential Sanitary Interest Present in Illicit Heroin

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Summary. The injection of heroin mixtures poses difficult problems in some cases to both pathologist and toxicologist in evaluating the cause of death. Direct heroin toxicity, allergic or idiosyncratic reactions, and bacterial and mycotic infections are different complications that can be found in these cases. The mycotic profile (mycoflora) present in illicit heroin from street samples (sachets) has been studied. The profile was obtained after incubating the samples at pH5 in 2% and 3% agar-maltose media. Only two samples (nos. 24 and 26) yielded negative results. Twenty-seven taxa were isolated. *Penicillium* and *Aspergillus* were the genera most frequently found in the samples. Other genera, such as *Cladosporium, Absidia, Rhizopus* and *Micelia sterilia* were also detected. Most of the fungi isolated presented a high allergenic potential and were liable to result in allergic reactions connected with "drug deaths". All the fungi found were commonly present in our environment.

Key words: Illicit heroin, street samples – Heroin and mycotic profile – Heroin mycoflora – Heroin, fungi

Zusammenfassung. Die Injektion von Heroin wirft in einigen Fällen schwierige Probleme für den Pathologen und den Toxikologen auf, wenn es um die Klärung der Todesursache geht. Direkte Heroin-Toxizität, allergische oder idiosynkratische Reaktionen und bakterielle und mykotische Infektionen sind verschiedene Komplikationen, welche wir in diesen Fällen finden können. Das mykotische Profil (Mycoflora), das in illegalen Heroinproben (Sachets) vorhanden ist, wurde untersucht. Das Profil wurde nach Inkubation bei pH5 in 2% oder 3% Agar-Maltose Medium erhalten. Nur zwei Proben hatten negative Befunde. Siebenundzwanzig „taxa" wurden isoliert. *Penicillium* und *Apergillus* waren die am häufigsten gefundenen Stämme. An-

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dere, wie *Cladosporium, Absidia, Rhizopus* und *Micelia sterilia* wurden ebenfalls nachgewiesen. Die meisten isolierten Pilze präsentierten ein hochallergenes Potential und waren imstande, allergische Reaktionen auszulösen, welche mit Drogen-Todesfällen verbunden sind. Alle nachgewiesenen Pilze waren im allgemeinen in unserer Umgebung vorhanden.

Schlüsselwörter: Heroin, Straßenproben – Heroin und mykotische Profile – Heroin-Mycoflora – Heroin, Pilze

Introduction

The incidence of death following injection of heroin mixtures poses difficult problems to both pathologist and toxicologist in evaluating the true cause of death. Direct heroin toxicity, allergic or idiosyncratic reactions, and bacterial and mycotic infections are different complications that can be present either alone or in combination.

Heroin addicts very frequently undergo infectious complications, the most common of which are endocarditis, hepatitis, pneumonia and phlebitis. Infections brought about by fungi and yeast, although much less frequent than those caused by bacteria, have been reported extensively in the literature over the last few years.

The ease with which the spores and mycelia of fungi spread throughout the environment and the weakening of the immunologic response of heroin addicts favor the development of such infections, aided by the use of lemon, or less often vinegar, to facilitate the dissolution of powdered heroin, which results in an acidification of the medium that fosters the growth of fungi and yeasts, as well as by the habit of sucking the needle prior to the venous puncture, thus contaminating the injection with the fungi and yeasts present in the mouth.

Fungi and their spores may work their way into the samples in the manufacture, storage, transport, and manipulation stages. Consequently, a given sample will contain various fungi and spores according to the medium and the subjects who manipulated it. Saint Jean et al. (1983) postulated a relationsship between fungus-induced infections and the type of heroin consumed.

Materials and methods

We have studied 43 sachets of heroin from 29 different batches seized by the Spanish police in Granada. Of the 43 samples, 38 were of the so-called "brown sugar" variety, and the remainder corresponded to the white heroin kind.

To allow the growth of the spores of the viable fungi presumably present and to avoid contaminating the samples through manipulation as far as possible, these were inoculated directly, using 2.3–36 mg (average weight 16.254 mg; SD 10.403). The inoculation was carried out in disposable, sterile Petri dishes (diameter 120 mm), using two universal media for mycologic culture which allowed the growth of a broad spectrum of filamentous fungi and yeasts present in the environment. Afterward, the dishes were incubated in a stove at 27°C for 12–14 days, subsequently counting and determining the colonies developed.

The composition of the culture media employed was as follows:

Agar-Glucosated 2%-Malt Extract		Agar-3% Malt Extract	
Malt extract	20 g	Malt extract	30 g
Glucose	20 g	Mycologic peptone	5 g
Mycologic peptone	1 g	Agar	15 g
Agar	25 g	Distilled water	1000 ml
Distilled water	1000 ml		

Both media were adjusted to pH5 and sterilized for 20 min in an autoclave at 1 atm and 120°C prior to use.

Table 1. Average of the colonies developed in all the samples

Taxa	Colonies developed		Occurrences	
	No.	(%)	No.	(%)
Absidia corymbifera (Cohn) Sacc. & Trotter	1	0.6	1	1.2
Acremonium kiliense Grütz	3	1.8	1	1.2
Aspergillus candidus Link	14	8.4	1	1.2
Aspergillus fumigatus Fresenius	32	19.3	11	13.1
Aspergillus niger V. Tiegh.	1	0.6	1	1.2
Aspergillus proliferans Smith	13	7.8	8	9.5
Aspergillus sydowi (Bainier & Sartory) Thom & Church	1	0.6	1	1.2
Aspergillus versicolor (Vuill.) Tiraboschi	15	9.0	5	5.9
Aspergillus alliaceus Thom & Church	3	1.8	3	1.6
Cladosporium cucumerinum Ellis & Arth.	9	5.4	7	8.3
Cladosporium oxysporum Berk. & Curt.	1	0.6	1	1.2
Monilia sitophila Mont.	10	6.0	8	9.5
Mucor indicus Lendner	1	0.6	1	1.2
Penicillium brevicompactum Dierckx	17	10.2	3	3.6
Penicillium chrysogenum Thom	3	1.8	2	2.4
Penicillium decumbens Thom	1	0.6	1	1.2
Penicillium fellutanum Biourge	3	1.8	2	2.4
Penicillium hispanicum Ramirez, Ferrer & Martínez	1	0.6	1	1.2
Penicillium implicatum Biourge	1	0.6	1	1.2
Penicillium puberulum Bain.	2	1.2	1	1.2
Penicillium waksmanii Zaleski	3	1.8	2	2.4
Rhizopus nigricans Ehrenb.	1	0.6	1	1.2
Scopulariopsis brevicaulis (Sacc.) Bainier	7	4.2	3	3.6
Syncephalastrum racemosum Cohn ex Schroeter	2	1.2	2	2.4
Micelia sterilia	18	10.8	13	15.5
Yeasts	3	1.8	3	3.6

Table 2. Results of the samples

Taxa	Sample No.																													
	1	3	6	7	8	10	11	13	14	15	16	17	18	19	21	22	23	24	25	26	27	28	29	31	32	33	34	35	36	Total
Absidia corymbifera													1																	1
Acremonium kiliense												3																		3
Aspergillus candidus									14																					14
Aspergillus fumigatus			2	10	1			2	3		1				1							7		4				1		32
Aspergillus niger			1																											1
Aspergillus proliferans	2						1				1	1			1									5	1	1				13
Aspergillus sydowi		1																												1
Aspergillus versicolor		4		2						1											1	7								15
Aspergillus alliaceus											1	1																	1	3
Cladosporium cucumerinum	1											3			1											1	2		1	9
Cladosporium oxysporum															1															1
Monilia sitophila			1						2	2				1		1	2							1						10
Mucor indicus																											1			1
Penicillium brevicompactum					1																	7		9						17
Penicillium chrysogenum																			1								2			3
Penicillium decumbens																													1	1
Penicillium fellutanum				2												1														3
Penicillium hispanicus				1																										1
Penicillium implicatum										1																				1
Penicillium puberulum									2																					2
Penicillium waksimanii										1				2																3
Rhizopus nigricans																							1							1
Scopulariopsis brevicaulis												1			1							5								7
Syncephalastrum racemosum													1														1			2
Micelia sterilia	1	1	1					1	3			1	1	3	3										1	1			1	18
Yeasts	1					1				1																				3
Bacterias		2				1	1			1						1	2		2		4	1			1		1			17
"Undeveloped"				3						3		2			4	1														13
Total	5	8	5	18	2	2	2	3	24	10	3	12	3	6	12	4	4	0	3	0	5	27	1	19	3	3	7	1	4	196

We have grouped the 43 sachets from 29 samples according to their origin. We have made the following grouping: Sample 6 included two sachets, sample 14 included four samples from four sachets, sample 16 included two sachets; the same applies to samples 17, 18, 22, 23, 32, and 34

Results

In Tables 1 and 2 are listed the different taxa identified, along with the number of colonies grown in each sample.

Only filamentous fungi have been identified; therefore, yeasts and bacteria appear within general groups since they were not considered in this study.

We have also established an "undeveloped" group, made up by those colonies which failed to grow sufficiently so as to be determined, owing to the inhibitory effect exerted upon their growth by other competing fungi present in the media.

Finally, the group of *Micelia sterilia* includes those fungi which did not develop reproductive structures (conidia, sporangiospores and ascospores) and could not be determined therefore.

Discussion

Only the cultures corresponding to four of the samples yielded negative results. All fungi detected in the samples are common in Spain, except for *Scopulariopsis breviculais* (Sacc) Bainier, which, although reported in our country, has never been detected in the south of the Iberian peninsula.

Fungi belonging to the genus *Aspergillus* Mich. ex Fries are represented by some species which are highly active in the production and storage of mycotoxins (Gravesen 1979). *Aspergillus fumigatus* Fresenius and *Aspergillus niger* V. Tiegh act as opportunistic pathogenic agents liable to cause bronchial asthma, bronchopulmonary allergy, extrinsic allergic alveolitis, aspergilloma, and invading aspergillosis, which frequently affects weakened tissues (e.g., burnt skin). Cole and Kendrik (1981) regard *Aspergillus fumigatus* Fresenius as the most common pathogenic agent of this genus. This species, which may grow saprophytically in the lung, either by means of a mycellium spread throughout the bronchi or by forming a compact sphere made up of hyphae (aspergilloma), can invade and attack other tissues in immuno-deficient humans, as well as cause asthma and pulmonary eosinophilia in patients with bronchopulmonary aspergillosis (Gregory 1973; Deacon 1984). The massive inhalation of conidia from this fungus results in alveolitis and crises of asthma, or even in allergic reactions in sensitive individuals (Bernton 1930; Brown 1932; Gregory 1973; Deacon 1984). The conidia of this fungus may also cause hypersensitivity reactions type I and II (Muñoz Lopez and Martin Mateos 1983).

Raper and Fenell (1965) attributed the intoxicating effect of oxalate from foodstuff to *Aspergillus niger* V. Tiegh.

Serious intoxications provoked by *Absidia corymbifera* (Cohn.) Saco and Trotter, give rise to intestinal processes which may result in gastric and intestinal perforations or even in the invasion of intestinal vessels and their clogging (Cole and Kendrik 1981). *Monilia sitophila* Mont. and *Penicillium chrysogenum* Thom are liable to induce positive skin reactions upon cutaneous injection (Brown 1936).

Some species belonging to the genus *Cladosporium* Link. ex Fries give rise to the appearance of non-seasonal conjunctivitis (Muñoz Lopez and Martin Mateos 1983).

As stated in the Introduction, the prior acidification introduced by lemon juice or related substances favors the development of fungi from their spores. However, most of the references reported in the literature to date deal with infections caused by yeasts *Candida albicans)* which, rather than being present in the heroin sample itself, we only found yeasts in three samples, would be introduced by carriers of oral aphthae in the manipulation preceding consumption, aided by the occasional use of lemon, which plays an important role as supporting medium according to Newton-John et al. (1984).

As potential infectious agents, fungi must compete with numerous yeasts and bacteria present in the associated flora; this may account for the short number of references available and fungi isolated in infectious processes, such as mycotic aneurisms, frequently undergone by heroin addicts. In a study involving 28 cases of mycotic aneurism in pulmonary arteries, Navarro et al. (1984) could only isolate *Actinomyces* and *Aspergillus* in one case each, the associated flora most frequently found consisting basically of staphylococci.

We believe that their pathogenic potential in the most frequent complications suffered by heroin addicts is not infectious; rather, owing to their high sensitizing power, many suspected "overdoses" with morphine blood levels under those considered lethal would be related to allergic reactions, with their associated shock symptoms. In this sense, it should be noted that most of the fungi isolated by out team are powerful sensitizers; on the other hand, owing to their ubiquity in the environment, they would be scarcely useful as possible tracers in the identification of different batches.

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